Estimated exposure to hepatitis E virus through consumption of swine liver and liver sausages

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Abstract: A quantitative risk assessment was undertaken following the Codex Alimentarius principles in order to predict the exposure of consumers to hepatitis E virus (HEV) through food consumption. Taking into account the tropism of HEV, fresh liver and liver sausages were regarded as having a higher risk of contamination. The model entailed a hypothetical food pathway and was based on worst case scenario where the intake of contaminated food derived from a 100% HEV-infected pig population was estimated. As no data on the prevalence of infectious HEV was available, the HEV-RNA prevalence in food matrices and the seroprevalence of HEV-specific antibodies in swine were assessed and adjusted for diagnostic misclassification and sampling uncertainty. Considering a HEV prevalence of 100% in pigs and excluding further cross-contamination events, a food portion consisting of 130 gr of liver or of 32.5 gr of sausage (containing 30% of liver) yielded an estimated exposure of 8047 and 210 RNA copies (median values), respectively. These findings take into account the effect of thermal treatment on the HEV-RNA concentration of food. Due to the lack of information concerning the correlation between HEV-RNA concentration and the amount of infectious virus as well as the dose-response relationship of HEV, the calculated RNA copies do not allow direct conclusions to be drawn on the risk of infection following ingestion of these food types. The true prevalence was estimated for Switzerland and Germany, leading to an overall prevalence of HEV-RNA in food of 6.2% (90% Highest Density Intervals (HDIs): 2.5%–11.2%). In comparison with fresh liver, liver sausages showed a higher prevalence, most likely due to the presence of more than one liver within the same sausage. The true prevalence of anti-HEV IgG ranged between 59.4% (HDIs 56.5%–62.4%) and 62.6% (HDIs 58.8%–64.3%) and between 7.6% (HDIs 3.3%–13.2%) and 30.5% (HDIs 23.2%–38.2%) in pigs and wild boars, respectively. The high prevalence of antibodies support the evidence that these animals can act as reservoirs for HEV and can contribute epidemiologically to the maintenance of the virus in the surroundings. This study is a preliminary investigation and highlights the major existing gaps needed to be filled in order to enable a refined HEV risk assessment that can drive future decisions for the implementation of food safety and of control measures.

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Estimated exposure to hepatitis E virus through consumption of swine liver and liver sausages

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Abstract

A quantitative risk assessment was undertaken following the *Codex Alimentarius* principles in order to predict the exposure of consumers to hepatitis E virus (HEV) through food consumption. Taking into account the tropism of HEV, fresh liver and liver sausages were regarded at-higher risk of contamination. The intake of food originating from asymptomatically infected pigs was supposed. As no data on the prevalence of infectious HEV was available, the HEV-RNA prevalence in food matrices and the seroprevalence of HEV-specific antibodies in swine were assessed and adjusted for diagnostic misclassification and sampling uncertainty. Considering a HEV prevalence of 100% in pigs and excluding further cross-contamination events, a food portion consisting of 130 gr of liver or of 32.5 gr of sausage (containing 30% of liver) yielded an exposure of 8047 and 210 RNA copies (median values), respectively. These findings take into account the effect of thermal treatment on the HEV-RNA concentration of food. Due to the lack of information concerning the correlation between HEV-RNA concentration and the amount of infectious virus as well as the dose-response relationship of HEV, the calculated RNA copies do not allow direct conclusions on the risk of infection and disease that follows ingestion of these food types. The true prevalences were estimated in Switzerland and Germany, leading to an overall prevalence of HEV-RNA in food of 6.2% (90% Highest Density Intervals (HDIs): 2.5%-11.2%). In comparison with fresh liver, liver sausages showed a higher prevalence, most likely due to the presence of more than one liver within the same sausage. The true prevalence of anti-HEV IgG ranged between 59.4% (HDIs 56.5%-62.4%) and 62.62% (HDIs 58.8%-64.3%) and between 7.6% (HDIs 3.37%-13.2%) and 30.5% (HDIs 23.2%-38.2%) in pigs and wild boars, respectively. The high rates of antibodies support the evidence that these animals can act as reservoirs for HEV and can contribute epidemiologically to the maintenance of the virus in the surroundings. This study is a preliminary investigation highlighting the major existing gaps

needed to be filled in order to enable a refined HEV risk assessment that can drive future decisions for the implementation of food safety and of control measures.

Keywords

Hepatitis E virus, exposure assessment, liver, liver sausages, foodborne transmission

1. Introduction

Hepatitis E virus (HEV) is the causative agent of an acute and self-limiting hepatitis and is commonly transmitted via the fecal-oral route (Pavio and Mansuy, 2010; Bonney *et al*., 2012; Emerson and Purcell, 2003). Belonging to the *Hepeviridae* family (Emerson *et al*., 2004), HEV is a non-enveloped positive-stranded RNA virus (Emerson and Purcell, 2003), which is classified into four major human pathogenic genotypes with different host ranges and geographical distribution. HEV genotypes 1 and 2 are found exclusively in humans while genotypes 3 and 4 have been detected also in animals and pigs and wild boars are considered the main reservoirs (Meng, 2010; Pavio and Mansuy, 2010; Meng, 2011).

Swine HEV infection is usually subclinical; pigs show no overt disease signs or pathological lesions. Pigs are normally infected at the age of 4-8 weeks resulting in a transient viremia and short fecal shedding (Pavio *et al*., 2010). In Europe, seroprevalence rates (anti-HEV IgG) indicating previous HEV infection range between 58.8% and 71.3% in fattening pigs (Burri *et al*., 2014; Wacheck *et al*., 2012a) and between 12.5% and 41.3% in wild boars (Burri *et al*., 2014; Schielke *et al*., 2015).

In humans, clinical symptoms of hepatitis E are indistinguishable from other forms of acute hepatitis (Purcell and Emerson, 2001). The case fatality rate among patients is generally below 1-5% (Pavio *et al*., 2010), with the exception of pregnancy where rates up to 25% have been reported (Kumar *et al*., 2004).

Although large outbreaks of hepatitis E seem to be confined to low-income countries as a consequence of poor water hygiene conditions, sporadic cases are reported globally, including Europe. In Switzerland, hepatitis E is not notifiable; therefore the exact number of cases is unknown. Nevertheless, autochthonous cases were diagnosed in 2004 (Sudre *et al.*, 2005) and in 2013 (Hiroz *et al*., 2013). Cases have been also reported after exposure to game meat (Joller and Gaudenz, 2015). Recently, in Germany, the number of notified hepatitis E cases has risen steeply. In 2014, 670 cases were reported with an increase of 46% compared to 2013 (Robert Koch Institute, 2015). Antibodies against HEV have been found in both the general population (Schnegg *et al*., 2013; Dremsek *et al*., 2012) and - with increased prevalence - in individuals with occupational exposure to swine and wild boars (Wilhelm *et al*, 2011; Schielke *et al*., 2015; Krumbholz *et al*., 2014). The foodborne transmission of HEV has been described in Japan and in France reporting the presence of genetically related strains in both the food and the patient after the ingestion of contaminated game meat, wild boar and pig meat, or pig liver sausages (Tei *et al*., 2003, Colson *et al*., 2010, Colson *et al*., 2012).

In order to estimate the exposure of an individual to HEV through the consumption of food, a quantitative risk assessment was attempted following the *Codex Alimentarius* Commission principles. Input data for the development of the present study were obtained from the scientific literature. The model entailed a hypothetical food pathway and was based on a worst-case

87 scenario where the intake of contaminated food derived from a 100% HEV-infected pig
88 population was assumed.

89 **2. Materials and Methods**

90 The overall scenario pathway for this quantitative exposure assessment to HEV via the
91 consumption of food is represented with a three-module structure (Figure 1). Two extra modules,
92 that are not part of the food pathway, are also described in order to provide a broader view of the
93 available knowledge of HEV.

94 2.1. Literature search

95 A review of the literature was performed to identify studies describing the HEV-RNA
96 concentration in fresh liver and liver sausages (module 1). Only studies that quantified the RNA
97 amount by real time PCR (RT-qPCR) per each of the positive samples were included. A search
98 was also carried out to identify studies reporting the effect of the thermal treatment on RNA
99 concentration (module 2). Studies in which the logarithmic reduction of a given viral load was
100 measured were taken into account. Supplementary research on the prevalence of HEV-RNA in
101 food was done (module a). The module aimed at the estimation of the prevalence (referred to as
102 "true prevalence" from now on) and was attempted through adjustment for diagnostic
103 misclassification and quantification of sampling uncertainties. Prevalence studies from
104 Switzerland and Germany were eligible. The current knowledge on the seroprevalence of
105 antibodies against HEV in pigs and wild boars was also reviewed (module b) with the aim of
106 estimating the true prevalence of anti-HEV IgG in swine. Selection criteria were as follows:
107 prevalence data obtained from commercial ELISA assays intended for serum and meat juice
108 samples; studies carried out in the abovementioned countries within the last ten years. In addition

109 to studies selected from the literature review, unpublished serological data from a one-year Swiss
110 project were included (Table 4).

111 2.2. Initial concentration of HEV-RNA in food (module 1)

112 Following the previous selection criteria, a total of three and two studies were included in order
113 to evaluate the initial concentration of HEV-RNA in fresh pig liver and liver sausages,
114 respectively. Table 1 shows the number of positive samples and the corresponding titers.
115 Concentrations were first converted into the same unit of measure (number of genome copies/gr)
116 and then transformed into $\log_{10}$ copies/gr. In the study from Leblanc *et al*. (2010), the viral RNA
117 titer per each positive liver sample was expressed as a range of values falling in the same log
118 level. Thus, to gain a final value (in $\log_{10}$ copies/gr), a uniform distribution was applied with
119 minimum and maximum values as observed in the studies. Thereafter, samples whose titers fell
120 in the same $\log_{10}$ level were grouped and their relative and cumulative frequency calculated. The
121 initial concentration of HEV-RNA was estimated using a cumulative distribution as described by
122 Vose (1996). The initial concentration in liver sausages took into account the proportion of liver
123 (on average 30%, modeled by Pert distribution) in the final product. Table 5 shows an overview
124 of the distributions that were used in the present assessment. The software package @RISK™
125 version 5.5 (Palisade, Newfield, NY) for Excel™ (Microsoft Corp., CA) was used with ten
126 thousand iterations and one simulation for all distributions. Bayesian analysis was used to reduce
127 the uncertainty around the predicted exposure estimate.

128 2.3. Effect of thermal treatment (module 2)

129 To investigate the effect of thermal treatment on HEV-RNA concentration in fresh pig liver and
130 liver sausages, a total of two and one studies were chosen, respectively (Table 2). Load

reductions were grouped based on three core-temperature ranges: up to 60°C (resembling rare-medium cooking), up to 69°C (medium-medium well), and above 70°C (well done). For each temperature range, a time range between 1 and 15 min was considered (Table 2). A Pert distribution was used to model the mean HEV-RNA log reduction (Log_{10}copies/gr) after thermal treatment (Table 5).

2.4. Final concentration of HEV-RNA in food (module 3)

The final concentration was defined as the number of viral RNA measured in Log_{10} copies/gr that remained within the food at the time of consumption quantified as the mean HEV-RNA load reduction after thermal treatment subtracted from the initial HEV-RNA concentrations.

2.5. Outcome: Predicted exposure to HEV-RNA

The predicted exposure of humans to HEV was defined as the ingested viral RNA copy number per single serving size. The mean amount of liver and liver sausages constituting a serving size relied on information from the BLS – Bundeslebensmittel Schlüssel (Max Rubner-Institut, 2010) database and was modeled with Pert distributions (Table 5). Subsequently, the final concentrations and the serving sizes were combined in a Poisson distribution (Table 5) with the assumption that the virus was randomly distributed throughout the food matrix.

A sensitivity analysis based in correlation analysis was conducted to identify and to rank the inputs that most significantly affect the final outcome.

2.6. True prevalence distribution of HEV-RNA in food (module a)

A total of four studies were selected with the aim of investigating the true prevalence of HEV-RNA in liver and liver sausages. Table 3 illustrates the apparent prevalence of the virus RNA in

152 samples collected at slaughter and retail level. The total (pooled) apparent prevalence was given
153 by the proportion of the total number of positive samples and the total number of sample sizes.
154 From this, the Beta-distribution was derived (Table 5) and adjusted using the performance of the
155 diagnostic test in terms of sensitivity and specificity (diagnostic misclassification). Due to the
156 lack of validation studies, the test performances relied on the 95% sensitivity and 97% specificity
157 originated from a ring trial run on twelve samples repeated nine times. This test was based on the
158 RT-PCR described in Szabo *et al*. (2015). Assuming that the other three assays had the same
159 performance, the true prevalence (with 95% HDIs) was estimated using the BayesPeM web
160 application (Flor, 2016). The 95% HDI denotes the range of prevalence estimates that together
161 account for 95% of the probability mass of the distribution. Any value outside the 95% HDI has
162 less probability than the values inside it. Therefore, the HDI constitutes a natural measure of
163 uncertainty for the estimates (Flor, 2016).

164 2.7. True prevalence of anti-HEV IgG in swine (module b)

165 Three studies (one of which was unpublished) were selected as representative of the HEV-
166 specific antibody prevalence in fattening pigs in Switzerland. The type of samples, apparent
167 prevalence, CLs, and test features are given in Table 4. The total apparent prevalence and the
168 relative Beta distribution were calculated as previously described. Prior sensitivity and specificity
169 were defined as Beta distributions based on validation studies (Table 5). With regard to Swiss
170 wild boars, only one study was available. The true prevalence was estimated as described above.

171 Data on HEV-specific antibody prevalence in pigs in Germany were extracted from three studies
172 (Table 4) and the estimation of true prevalence proceeded as described above. The apparent
173 prevalence was adjusted by the combination of the parameters originated from the two validated
174 diagnostic tests (Table 5). Two studies were considered regarding the German wild boars (Table

175 4). Sensitivity and specificity estimates for wild boar derived from studies of van der Poel *et al*.
176 (2014) and Herremans *et al*. (2007) (Table 5).

177 **3. Results and discussion**

178 3.1. Initial concentration, effect of thermal treatment and final concentration of HEV-RNA in
179 food

180 Estimation of the initial concentration of HEV-RNA in pig liver and liver sausages yielded mean
181 values of 4.15 and 3.7 Log_{10} copies/gr, respectively. The lower load in liver sausages in
182 comparison to liver could be due to a dilution effect occurring as result of the presence of other
183 ingredients in addition to plain liver.

184 These concentrations, which were estimated based on the viral RNA loads detected only in
185 positive samples, reflect the worst-case scenario of 100% HEV-infected pigs and therefore lead
186 to an overestimation. This approach was chosen due to the lack of data on the limit of detection
187 (LOD) of the performed molecular assays. This information was available only in the study from
188 Leblanc *et al*. (2010) where 34 samples were classified as negative if they fell below 10^3 RNA
189 copies/gr as the given LOD. After conducting a censored analysis using R-package NADA with
190 the function cenros (Helsel, 2012), a mean initial concentration of 2.63 Log_{10} copies/gr of fresh
191 liver was calculated. However, this represents a major point of uncertainty as the proportion of
192 identifiable values is low (21%) and the LOD is very high. Approximately 80% of the values are
193 censored (<LOD). The use of the censored method is not recommended when more that 80% of
194 values in a dataset are censored (Helsel, 2012). The mean, median, standard deviation and
195 quartiles estimates are rather unreliable.

196 Table 7 shows the identified factors that increase the uncertainty and variability of the present
197 assessment.

198 The effect of temperature in terms of RNA reduction resulted in a similar mean value of 2.27 and
199 2.22 Log_{10} copies/gr of liver and liver sausages, respectively. These results suggest that the
200 influence of temperature on the reduction of HEV-RNA load is not influenced by the food matrix
201 or proportion of liver in the liver sausage. However, it should be considered that the thermal
202 treatment data used for this assessment came from experimental studies carried out in laboratory
203 settings, which may differ from those in food production. For example, liver suspension and
204 infected cell lines were used in the studies from Barnaud *et al*. (2012) and Johne *et al*. (2016) in
205 order to investigate the HEV-RNA decline or infectious virus decline at specific combinations of
206 time and temperature. In the absence of information, extracted data from those studies were used
207 as surrogates to develop the present food model. Further studies are therefore needed to ensure a
208 more realistic approach in performing the HEV risk assessment and to reduce this major point of
209 uncertainty (Table 7).

210 To calculate the number of virus-RNA copies within the food at the time of consumption (final
211 concentration), the predicted RNA load reductions were subtracted from the initial
212 concentrations, resulting in values of 1.88 and 1.48 Log_{10} RNA copies/gr of liver and liver
213 sausages, respectively. These results were used as inputs for the model outcome.

214 3.2. Outcome: Predicted exposure to HEV-RNA

215 The consumption of a single serving consisting of 130 gr of liver and 32.5 gr of liver sausages
216 yielded an exposure to 8047 and 210 viral RNA copies (median values), respectively. When
217 censored data based on the study of Leblanc *et al*. (2010) were considered, a sensible reduction of

218 the number of copies (287 copies) per serving of plain liver was observed (Figure 2). These
219 findings represent the amount of ingested HEV-RNA copies by the general consumer if 100%
220 prevalence (all livers originated from infected pigs) is assumed. Stratification for gender, health
221 status or age groups and modeling of food intake frequencies for consumer group have not been
222 considered due to sparsity of the data.

223 The risk of developing human hepatitis is the probability of acquiring the infection from a given
224 exposure dose. Two main factors affect this probability: the level of exposure to the amount of
225 infectious HEV and the following interaction of HEV with the host (dose-response). The rather
226 complete lack of information in literature in view of these two factors limits the interpretation of
227 our assessment. First, the measurement of viral RNA (as applied in our assessment) does not
228 necessarily correspond to the amount of infectious virus present in a sample. However, so far
229 only data on the presence of HEV-RNA are available for pig liver and liver sausages. Future
230 investigations based on assays that are able to distinguish between infectious and non-infectious
231 HEV are necessary. To this end, novel methods for measurement of HEV infectivity, such as
232 efficient cell culture systems (Johne *et al*., 2016), should be developed. In addition, the amount of
233 infectious HEV needed to trigger infection and the disease in the host is still unknown. Therefore,
234 the dose-response relationship of the HEV infection needs to be investigated. Another limiting
235 factor of our assessment is that only few studies have been published on the detection of HEV in
236 pork products and the reported rates are difficult to compare due to the application of different
237 detection methods. Standardized protocols with documented efficiency and sensitivity should be
238 developed and applied in future.

239 In contrast to bacteria, which are free-living, viruses need living cells for their propagation and
240 cannot multiply in food. Thus, the growth effect during food processing and storage at retail level

241 was not considered relevant for the present model. Additionally, this model was driven under the
242 assumption that food is manufactured following the good hygiene and manufacturing practices to
243 avoid any cross-contamination of fecal origin. Contamination during slaughtering was considered
244 to play only a minor role because shedding has been more widely reported in younger animals
245 (Pavio *et al*., 2010); this study considered fattening pigs, which are typically between six and
246 nine months of age. Therefore, in our model, HEV contamination was solely attributed to
247 infection of animals.

248 The present assessment focused on pig liver and sausages containing liver because this organ is
249 considered the primary site of HEV replication and several small outbreaks of hepatitis E have
250 been linked to consumption of these products (Yazaki *et al*., 2003; Colson *et al*., 2010). The
251 estimates exposure should not be extrapolated to meat and meat products. Additional studies are
252 required in order to estimate the concentration in pork considering that meat is likely consumed
253 in larger quantities and with more frequency in comparison with liver products.

254 As result of the sensitivity analysis, the initial concentration of HEV-RNA in fresh liver was the
255 parameter (correlation coefficient 0.94) having the greatest impact on the final exposure. When
256 censored data were considered, a similar coefficient (0.92) was found. The load reduction after
257 thermal treatment was the second most relevant input; serving size had only a weak influence on
258 the final output.

259 3.3 True prevalence of HEV in food

260 The estimated overall true prevalence of HEV-RNA in porcine-derived food in both countries
261 was 6.2% (90% HDIs 2.5%-11.2%). This estimate derived from the combination of four selected
262 studies, whose individual mean simulated values and relative 90% DIs are given in Table 6. In

263 comparison with livers, liver sausages showed a much higher prevalence. This was interpreted as
264 a consequence of the manufacturing process, where livers from several infected pigs can become
265 part of the same end-product. Due to the lack of PCR validation data, the molecular assay
266 performances from one study were extrapolated to the other three studies.

267 3.4. Estimation of true prevalence distribution of anti-HEV IgG

268 An estimated true prevalence of 59.4% (HDIs 56.5% - 62.4%) based on the presence of anti-HEV
269 IgG in Swiss domestic pigs was obtained by quantitative modeling of results from three available
270 primary studies (Table 6). With regard to Swiss wild boars, a mean value of 7.3% (HDIs 3.37%-
271 13.2%) was derived from the paper of Burri *et al*. (2012). A slightly higher mean prevalence of
272 61.62% (HDIs 58.8% - 64.3%) was observed in the German domestic pig population (Table 6).
273 With regard to German wild boars, a mean value of 30.5% (HDI 23.2-38.2%) was described
274 (Table 6). The high anti-HEV IgG prevalence in serum or juice samples represents indirect
275 evidence that a high proportion of these animals have been infected with the virus. Therefore,
276 they can act as reservoirs and contribute to the maintenance of the virus in epidemiological
277 cycles. As antibodies are only indirect indicators of infection, no direct conclusion can be drawn
278 on the probability of animals to be shedders or to derive a contaminated food. Antibody
279 prevalence data derived using validated assays constitute the majority of the information on HEV
280 infections in animals available in literature. These assays are mainly based on IgG detection,
281 which alone, can only be used to indicate viral exposure rather then active infection. There is a
282 lack of knowledge on the number of acutely infected (IgM anti-HEV) animals that are more
283 likely to deliver contaminated food. It would be desirable to have an estimate of the proportion of
284 IgG seropositive animals that are actively infected in order to more completely assess the risk
285 derived from consumption of undercooked, contaminated pork products.

Conclusion

In conclusion, this study estimated the predicted exposure to HEV-RNA via the consumption of pig liver and liver sausages based on data reported in the literature. The true prevalence of HEV-RNA in food matrices and of anti-HEV IgG in swine in Switzerland and Germany was also estimated.

The present model was limited to certain food types and should not implicate that consumption of pork meat could lead to the same exposure. A lack of several data was identified, which limit the conclusiveness of the estimation: (i) the correlation between RNA detection and the presence of infective virus is not known, and (ii) is not clear which amount of infectious virus is needed to trigger HEV human infection and the induction of disease. Consequently, it is not clear which level of infectivity reduction would be necessary to prevent the foodborne infection and the disease. This study represents a fist investigation on this topic and it identifies the most important gaps that need to be filled in order to enable a comprehensive HEV risk assessment.

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Yazaki, Y., Mizuo, H., Takahashi, M., Nishizawa, T., Sasaki, N., Gotanda, Y., & Okamoto, H., (2003). Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be foodborne, as suggested by the presence of hepatitis E virus in pig liver as food. *The Journal of General Virology*, 84, 2351-2357.

452 Table 1. Initial concentration of hepatitis E virus-RNA in food (module 1): selected studies

Reference (year)	Country	Matrix	No. Positive/ No. Tested (%)	RT-qPCR (log_{10}copies/gr min-max)	Detected Genotype
Liver					
Wilhelm *et al.* (2014)	Canada	Liver (Retail)	14/283 (4.9)	3.31 - 6.67	3, 4
Leblanc *et al*. (2010)	France	Liver (Slaughter)	9/43 (20.9)	3.50 -7.50	nr
Yazaki *et al*. (2003)	Japan	Liver (Retail)	7/363 (1.9)	2.00 - 7.00	3, 4
Liver sausages					
Barnaud *et al*. (2012)	France	Liver patè suspension	10/10 (100)	4.41 - 7.35	nr
Di Bartolo *et al.* (2015)	Italy	Liver sausages (Retail)	10/45 (22.2)	3.44 - 5.34	3

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454 Table 2. Effect of the thermal treatment on hepatitis E virus-RNA reduction in food (module 2):
455 selected studies

Reference (year)	Country	Matrix	Time (min)	Temperature (°C)	Log reduction
Liver					
Schielke *et al*. (2011)	Germany	Wild boar liver suspension	15	<60	0.59[a]
			1	<60	1.3 [a]
Johne *et al*. (2016)	Germany	Infected cell line	1 - 1.5	<69	2-2.6[b]
			1 - 1.5 - 2	≥70	2.9 - 3.6 - 3.9[c]
Liver sausages					
Barnaud *et al*. (2012)	France	Liver patè-like suspension	5 - 10	<60	1.19 - 1.83 [b]
			5 - 10	<69	2.26 - 2.28 [b]
			5 - 10	≥70	2.58 - 2.94 [b]

456 [a] min and max values from two different papers combined in a Uniform distribution; [b] min and max values from the same paper
457 combined in a Uniform distribution; [c] min, max, and most likely values from the same paper combined in a Pert distribution.

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459 Table 3. Apparent prevalence of hepatitis E virus-RNA in food (module a): selected studies

Reference (year)	Country	Origin	No. Positive	Sample Size	Prevalence (%)	95% CLs	
Liver							
Müller *et al*. (submitted)	Switzerland	Slaughter	2	160	1.25	0.1	4.4
Wenzel *et al*. (2011)	Germany	Retail	8	200	4	1.7	7.7
Liver sausages							
Szabo *et al*. (2015)	Germany	Retail	11	50	22	11.5	35.9
Carl *et al*. (2014)	Germany	Retail	17	61	27.9	17.1	40.8
Total			38	471	8.06	5.7	10.9

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463 Table 4. Apparent prevalence of anti-hepatitis E virus IgG in swine (module b): selected studies

Reference (year)	Country	Species (age in months)	Sample	No. Positive	Sample Size	Apparent Prevalence	95% CLs		ELISA (Se, Sp)[a]
Wacheck *et al*. (2012b)	CH	Pig (6)	Meat Juice	97	200	0.485	0.413	0.556	Prionics (91.0%, 94.0%) [a]
Burri *et al*. (2014)	CH	Pig (6-8)	Serum	1161	1999	0.580	0.558	0.602	Prionics (91.0%,94.0%) [a]
Unpublished data* (2013)	CH	Pig (6)	Serum	637	1147	0.555	0.526	0.584	Prionics (91.0%, 94.0%) [a]
Total				1895	3346	0.566	0.413	0.556	
Burri *et al*. (2014)	CH	Wild boar (12)	Serum	38	303	0,125	0,090	0,168	Prionics (91.0%, 94.0%) [a]
Krumbholtz *et al*.(2013)	DE	Pig (3-9)	Serum	306	796	0.384	0.350	0.419	Prionics (91.0%, 94.0%) [a]
Dremsek *et al*. (2013)	DE	Pig (nd)	Serum	623	898	0.693	0.662	0.723	Prionics (91.0%, 94.0%) [a]
Wacheck *et al*. (2012a)	DE	Pig (6)	Serum	368	516	0.713	0.672	0.751	Mikrogen (96.6%, 97.1%) [a]
Total				1297	2210	0.586	0.566	0.608	
Schielke *et al*. (2015)	DE	Wild boar (nd)	Serum	19	46	0.413	0.269	0.567	Axiom (93.0%, 89.0%) [b]
Adloch *et al*. (2009)	DE	Wild boar (12)	Serum	32	107	0.299	0.214	0.395	Genelabs (97.0%, 98.0%) [c]
Total				51	153	0.333			

464 *Unpublished serological data from a one-year Swiss project run in 2013. Nd: not detected; [a] provided within manufacturer´s
465 instruction; [b] from van der Poel *et al*., 2014; [c] from Herremans *et al*., 2007.

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478 Table 5. Overview of the input parameters used in the present exposure assessment

Input	Distribution		Description	Unit	Source
Food					
$Prev_{True}$	BetaExpert(4.841;73.06)		True prevalence of HEV-RNA in liver and liver sausages based on 95% Se and 97% Sp (BayesPeM) [a]	%	Table 3; Se, Sp from the ring trial [b]
$Conc._{Init}$					
Liver	Cumulative(2;8;{2.3.4.5.6.7}; {0,033.0,3.0,4667.0,7333.0,8333.1})		Initial HEV-RNA concentration in food	Log_{10} copies/gr	Table 1
Liver sausages	Cumulative(2;8;{2.3.4.5.6.7}; {0.0,35.0,6.0,9.0,95.1})				
$Ther._{Treat}$					
	Liver	Liver sausages			
Rare-medium rare	Uniform(0.59;1.3)	Uniform(1.19;1.83)	Effect of the thermal treatment on the HEV RNA load reduction. (Values under the liver and liver sausages columns are further modeled with two Pert distributions)	Log_{10} copies/gr	Table 2
Medium-medium well	Uniform(2;2.6)	Uniform(2.26;2.28)			
Well done	Pert(2.9;3.6;3.9)	Uniform(2.58;2.94)			
Serving size	Pert(80;125;200)	Pert(25;30;50)	Ingested food per meal	gr	BLS [c]
$Liver_{Prop.}$	Pert(0.1;0.3;0.5)		Proportion of liver as ingredient in liver sausages	gr	
$Liver_{Consum.}$	Poisson($Liver_{Prop}$; Serving size)		Consumption of liver per serving size	gr	
Swine					
Switzerland					
$Prev_{App.}$_Pigs	Beta(1895+1;3346-1895+1)		Apparent prevalence of anti-HEV IgG in domestic pigs	%	Table 4
Se	Beta(332+1;365-332+1)		Estimated sensitivity based on the number of true positive and false negative (Prionics)	%	Table 4/Validation study
Sp	Beta(142+1;151-142+1)		Estimated specificity based on the number of true negative and false positive (Prionics)	%	Table 4/Validation study
$Prev_{True}$_Pigs	$Prev_{App.}$+(Sp-1)/Sp+(Se-1)		True Prevalence of anti-HEV IgG in domestic pigs	%	
$Prev_{App.}$_Wild boars	Beta(38+1;303-38+1)		Apparent prevalence of anti-HEV IgG in wild boars	%	Table 4/Validation study
$Prev_{True}$_Wild boars	$Prev_{App.}$+(Sp-1)/Sp+(Se-1)		True Prevalence of anti-HEV IgG in wild boars	%	
Germany					
$Prev_{App.}$_Pigs	Beta(1297+1;2210-1297+1)		Apparent prevalence of anti-HEV IgG in domestic pigs	%	Table 4
Se	Beta(417+1;453-417+1)		Estimated sensitivity based on the total number of true positive and false negative (Prionics+RecomLine)	%	Table 4/Validation studies
Sp	Beta(209+1;220-209+1)		Estimated specificity based on the total number of true negative and false positive (Prionics+RecomLine)	%	Table 4/Validation studies
$Prev_{True}$_Pigs	$Prev_{App.}$+(Sp-1)/ Sp+(Se-1)		True Prevalence of anti-HEV IgG in domestic pigs	%	
Se	Beta(474;25.3)		Estimated sensitivity based on combined tests (Axiom and Genelabs) with lower and upper estimates set at 93% and 97% (BayesPeM) [c].	%	van der Poel *et al*., 2014; Herremans *et al*., 2007
Sp	Beta(144;10.4)		Estimated specificity based on combined tests (Axiom and Genelabs) with lower and upper estimates set at 89% and 98% (BayesPeM) [b].	%	
$Prev_{True}$_Wild boars	BetaExpert(30.6;70.1)		True Prevalence of anti-HEV IgG in wild boars	%	

479 [a] BayesPeM web application ; [b] Ring trial based on the RT-PCR described in Szabo *et al*. (2015); [c] Bundeslebensmittel Schlüssel database.

480 Table 6. Estimated true prevalence of hepatitis E virus-specific antibodies in swine and viral
481 RNA in food.

Reference (year)	Country	Species/Food	Measurements	Mean Value (%)	90% Highest Density Intervals (%)	
Wacheck *et al*. (2012b)	CH	Pig	Anti-HEV IgG	49.8	42.5	57.0
Burri *et al*. (2014)	CH	Pig	Anti-HEV IgG	61.2	57.9	64.4
Unpublished Data (2013)	CH	Pig	Anti-HEV IgG	58.1	54.5	61.9
Burri *et al*. (2014)	CH	Wild boar	Anti-HEV IgG	7.3	3.37	13.2
Krumbholtz *et al*.(2013)	DE	Pig	Anti-HEV IgG	37.9	33.5	42.1
Dremsek *et al*. (2013)	DE	Pig	Anti-HEV IgG	74.5	70.7	78.5
Wacheck *et al*. (2012a)	DE	Pig	Anti-HEV IgG	73.5	68.9	78.5
Schielke *et al*. (2015)	DE	Wild boar	Anti-HEV IgG	37.5	23.8	52.2
Adloch *et al*. (2009)	DE	Wild boar	Anti-HEV IgG	29.7	22.4	37.6
Müller *et al*. (submitted)	CH	Liver	HEV-RNA	1.4	0.15	3.69
Wenzel *et al*. (2011)	DE	Liver	HEV-RNA	3.1	0.7	7.02
Szabo *et al*. (2015)	DE	Liver sausages	HEV-RNA	21.5	10.7	34.5
Carl *et al*. (2014)	DE	Liver sausages	HEV-RNA	27.5	16.3	40.1

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483 Table 7. Identified uncertainty and variability factors affecting the hepatitis E virus exposure
484 assessment study

	Uncertainty	Variability
Module 1		
Initial concentration of HEV-RNA	Small number of available studies and sample sizes. No country –specific data for Switzerland or Germany. Results based on positive samples (no LOD* available). The use of cumulative distribution, (based on log level).	Not considered due to lack of data
Module 2		
Effect of temperature on the HEV-RNA concentration	Small number of available studies and sample sizes. No country –specific data for Switzerland or Germany. Used of food surrogates and relative extrapolation to liver and liver sausages.	Not considered due to lack of data
Module 3		
Final concentration of HEV-RNA		Proportion of liver within a sausage may vary among recipes and countries.
Outcome		
Predicted Exposure		Age, gender, or health status and frequency of consumption not considered due to lack of data
Module a		
True prevalence of HEV-RNA in liver and liver sausages	Small number of available studies and sample sizes. Results based only on positive samples. No standard method for RNA detection.	Not considered due to lack of data
Module b		
True prevalence of anti-HEV IgG in pigs and wild boars	Based only on anti-HEV IgG ELISAs.	Not considered due to lack of data

485 *LOD= Limit of detection

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Figure 1. Tree-module structured food pathway for this quantitative exposure assessment to hepatitis E virus via the consumption of liver and liver sausages. For Switzerland and Germany, two extra modules are described, which are not part of the food pathway.

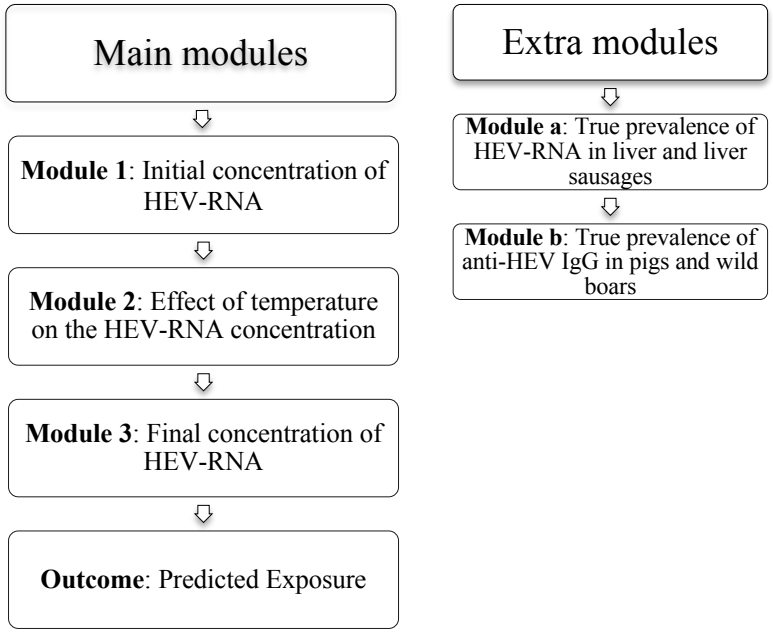

Figure 2. Predicted exposures to hepatitis E virus intended as ingested HEV-RNA copies per 130 gr of liver (black line) and 32.5 gr of liver sausages (dotted line). Censored data for liver are indicated with a grey line.